NOTES ON A CASE OF INDIGENOUS INFECTION WITH *P. FALCIPARUM*

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In their preliminary note on this case, Glynn and Matthews (1920) have furnished an account of the findings at the post-mortem examination and also details of the history. For convenience we may recapitulate here briefly the main facts.

History. The patient was a girl, aged 18, who had never been out of the British Isles; she was born in Liverpool and had once been South—to London in 1914, from July to September, during which period she spent three weeks at Littlehampton; she had lived for the last ten years in the same house in Liverpool. She died on October 12th, 1920, after a period of illness which commenced (on her return from a holiday in a northern health resort) with 'feverishness and flushed appearance' on October 1st, followed on October 2nd and 3rd and several other occasions by vomiting. On October 8th she was overcome with faintness in the street, and had to be helped home. Her condition rapidly became worse with severe headache, thirst, photophobia, delirium, paresis of legs, anuria, and finally coma. The temperature on October 11th, the day before death, was 100° F., and on October 12th, just before death, 102° F. Further investigation elicited the facts that she had always been pale, had suffered from headaches since Christmas, 1919, and that she had vomited on September 29th, 1920.

The following notes on the post-mortem examination are quoted from the paper mentioned above:—

'The post-mortem examination was made twenty hours after death. *Blood:* anaemic. *Brain:* apparently normal, no meningitis. *Lungs:* a moderate amount of oedema and muco-purulent bronchitis. *Heart:* weight, 8 oz., normal, no fatty degeneration. *Mouth:* healthy, no trace of pyorrhoea. Oesophagus, stomach and intestines opened from end to end—normal, no evidence of blood. Some of the lumbar lymphatic glands slightly enlarged, and red like haemolymph glands. *Liver:* weight, 3 lbs., slightly browner than normal, no haemosiderin reaction. *Spleen:* 1 lb. 7 oz., retains its shape, of normal consistency, but dark, rather like

malaria. Pancreas, suprarenals, kidneys and bladder normal. *Uterus:* normal size, but menstruating; one haemorrhagic corpus luteum. Bone marrow of the shaft of the femur a uniform terra-cotta red, that of the sternum redder and more succulent than normal.'

The following is a short account of my observations on the material examined. It is convenient to state here that the specimens from this case, which were demonstrated by me at a meeting of the Royal Society of Tropical Medicine and Hygiene, were by some inadvertence included under Col. James's exhibits, and erroneously stated to have been derived from a case in Sheerness. (See *Lancet*, January 1st, p. 26.)

Blood film. About a third of the red corpuscles contained young trophozoites, but owing probably to post-mortem changes the parasites appeared small and contracted, giving an appearance similar to that seen in sections. Not only was the total number of red cells infected large, but also multiple infection was common, many cells containing two, three, four, up to five parasites. Gametocytes were present in small numbers most of them being fully developed crescents, some of them presenting a peculiar appearance owing to the red cell being visible all round the parasite and extending some distance beyond it, and not chiefly in the concavity of the crescent; other forms were oval or spherical, and in addition there were seen solid-looking parasites of irregular outline which might be interpreted as developing forms of gametocyte. A small number of segmenting forms with a number of merozoites varying from ten to twenty-four also occurred in the blood, the crescents and fully divided forms being in about equal proportion.

Nucleated red cells were numerous, as was observed in the preliminary note, the figure given being 3·8 nucleated reds to 100 leucocytes (500 counted), while my figure is slightly higher, 4·9 to 100 leucocytes (1,000 counted). They presented much diversity in the form of the nucleus, single-nucleated forms predominating, but forms with double and multipartite nuclei also being found. Pigment was present in mononuclear leucocytes and also free in the plasma, some in the latter possibly being due to the breaking of segmenting forms in making the film.

Spleen smear. The chief feature to attract attention was the occurrence of very numerous segmenting forms, not all at the same

stage of sub-division, the merozoites numbering from eight to twenty-two. There were a few crescentic, oval, and spherical gametocytes. An occasional parasite, even in the segmenting stage, was found ingested by phagocytes. Pigment, both black and golden yellow, was present in considerable amount, ingested by large mononuclear white cells.

Bone marrow smear. Here crescentic and oval forms of gametocyte were numerous in quite different proportions to the numbers in either the blood or spleen smears. A few segmenting forms occurred and many small trophozoites, of which some were free. Among the large mononuclear cells, many were observed which contained eosinophil granules, and many also containing pigment.

Several questions of interest arise in connection with this case, and it is necessary, in spite of the limited character of the observations made on the case during the period of her illness and the small amount of material available for examination, to endeavour to answer some of the questions.

1. Was this a primary acute attack following directly the incubation period, or a secondary acute attack supervening in a person already infected for a considerable time?
2. By what means did the patient acquire infection?
3. At what time and where did she become infected?

I. WAS THIS A PRIMARY OR SECONDARY ACUTE ATTACK?

(*a*) *Primary acute attack.*

The absence of any history, previous to October 1st, 1920, of fever, shivering or sweating, is in favour of this being a primary acute attack.

Spleen. Support is also lent to this theory by certain appearances presented by the spleen. The noteworthy condition seen in section of this organ is the remarkable congestion, with dilatation of the sinuses. These facts are illustrated by photograph I, representing a section from the spleen of the case under discussion. That the large size of the spleen is due to a recent enlargement of the organ is further demonstrated when we observe that the increase of size is due to engorgement with blood and not to increase of fibrotic tissue, nor to the cellular elements of the spleen itself.

(*b*) *Secondary acute attack.*

It is quite possible for a person to harbour and present for considerable periods in his blood *P. falciparum* without the production of definite signs and symptoms, provided the person is taking quinine or other drugs. This fact has been observed frequently, and instances will be found in the work of Stephens and his collaborators (1917). Similarly it is recognised that persons suffering from relapses of malignant tertian malaria may have parasites present in the peripheral blood for varying periods before

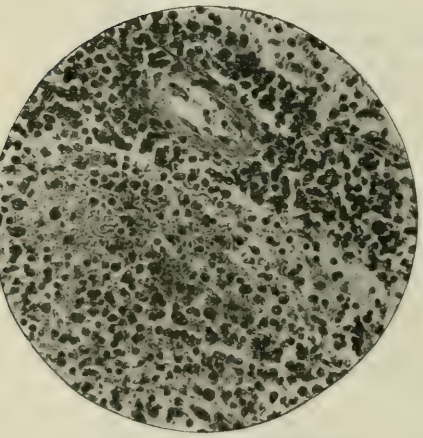

PHOTO. I. Section of the spleen showing the Malpighian body with vessel cut obliquely and below it the accumulation of red blood corpuscles. × about 280.

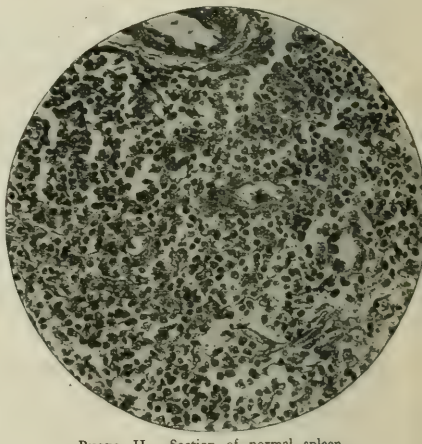

PHOTO. II. Section of normal spleen. × about 280.
Photographs lent by Professor Glynn

the next attack occurs. But it is also possible that a person who is infected may, even if untreated, remain without definite signs or symptoms for a long period and then suddenly develop an acute attack; instances of incubation periods up to 'months' are mentioned by Craig (1914). It should be observed, however, that in experimental infections in which healthy persons are injected with blood containing parasites or are bitten by anophelines containing sporozoites in their salivary glands, such long incubation periods are not recorded. Ross, whose contribution to the elucidation of

this case is dealt with more fully below, does not supply information which is of assistance concerning this point. In the case under consideration the only evidence of old standing symptoms or signs which might possibly be attributed to malaria are that the girl had suffered from headaches from Christmas, 1919, her pallor, and the fact that she vomited on September 29th. With regard to these phenomena, they may be attributed to so many other causes that they can give us no clue.

Spleen. The features which may be regarded as indicative of chronicity are the size (644 grms.) and lack of diffluence. As regards the former, Dudgeon and Clarke (1918-19) give the weight of the spleen in a series of fatal cases of pernicious malaria due to *P. falciparum* as varying from 250 to 450 grms.; the highest weight in a chronic case was 960 grms. On the other hand, James (1920) emphasises the great splenic enlargement observed in indigenous cases of simple tertian malaria in children. Previously Osler (1901) mentioned a case of death from accident during the second week of simple tertian malaria where the spleen weighed 800 grms., was very dark red, and diffluent.

II. BY WHAT MEANS DID THE PATIENT ACQUIRE INFECTION?

The two known methods by which it is possible to transmit malaria to a healthy person are the inoculation of infected blood and the transmission by the bite of a mosquito with infected salivary glands. The incubation period, using such methods, varies considerably for malignant tertian parasites, and it is necessary to enquire into the periods and how they are estimated.

The inoculation of Infected Blood into Healthy Persons

Of the numerous records obtained by inoculating blood containing malaria parasites into healthy persons only a small number is concerned with the malignant tertian parasite. Some of the early experiments collected by Thayer and Hewetson (1895) are, for comparative purposes, set out here in tabular form.

It will be seen from Table I that in the early experiments the parasite incubation was long. These early experiments are, however,

open to several objections. In Gualdi and Antolisei's first two experiments the inoculation of blood from quartan patients resulted in the appearance, in the subjects of inoculation, of malignant tertian parasites. The authors had to account for this by discovering later that the patient whose blood was inoculated harboured both forms of parasite. In the third case recorded by Gualdi and Antolisei, and that of Di Mattei, crescents only were visible in the blood injected, while in the last case, that of Sacharow, not only was the injection small in amount and given subcutaneously, but also the blood was taken from leeches which had been fed four days previously on the infected person and which, since feeding, had been kept on ice.

TABLE I.

Plasmodium falciparum. Incubation period following blood inoculation. Early experiments.

Observer	Route of Injection	Amount of Blood injected c.c.	Day on which, in inoculated person		Remarks
			Fever commenced	Parasites appeared	
Gualdi and Antolisei	Intravenous ...	3	10	10	Source of blood, quartan cases.
Gualdi and Antolisei	Intravenous ...	3	12	12	Source of blood, quartan cases.
Gualdi and Antolisei	?	2	9	10	Crescents only found in blood injected. Crescents seen in inoculated person in 18 days.
Di Mattei	Intravenous ...	?	16	In a few days after injection	Crescents only found in blood injected. Crescents seen in inoculated person in 25 days.
Sacharow	Subcutaneous	1	12	13	Blood kept in leeches on ice 4 days before injection.

Experiments were performed later by Bastianelli and Bignami, which are set out in Table II, and from this series a very different idea of the incubation period following inoculation is obtained. Parasites appeared in the peripheral blood in so short a period as two days after injection of 2 c.c. of infected blood, and infection resulted after such small quantities as 0·2 c.c. of blood injected. No mention is made as to the date of crescents appearing in the blood in these cases.

It appears, then, that the incubation period after experimental inoculation, whether as regards occurrence of fever or appearance of parasites in the blood, may be exceedingly brief in malignant tertian malaria.

Ross (1920) has suggested the possibility of this case having acquired infection by inoculation of blood. There was no evidence in the history of the case that such a mode of infection could have been the cause. She had cocaine injections for removal of teeth in July, 1919, and in February, 1920; for the removal of the teeth, mentioned as done on 14th September, 1920, the anaesthetic used was ether, and no evidence was obtained either that any such injections as morphia had been given, or that infection could have

TABLE II.

Plasmodium falciparum. Incubation period following blood inoculation. Later experiments.

Observer	Route of Injection	Amount of blood injected c.c.	Day on which, in inoculated person		Remarks
			Fever commenced	Parasites appeared	
Bastianelli and Bignami ...	Intravenous ...	2·0	2	2	
	...	5·0	2	2	Few parasites seen in injected blood.
	...	0·75	5	?	Few parasites seen in injected blood.
	...	0·2	4	?	Fair number of parasites seen in injected blood.

arisen from abrasions of skin or mucous membranes having facilitated transmission. It is regrettable that Ross, who has sources of information inaccessible to others, should not have communicated the facts in such a form as to have added to our knowledge of the various points of interest which must have arisen as the result of his observations. The incubation period of malignant tertian malaria under such circumstances, the dates of the first appearance of symptoms and parasites, and also the important question as to the time of the appearance of crescents, are all matters of sufficient interest to make welcome any additional facts relating to them.

Incubation period of P. FALCIPARUM *following the Bites of Infected Anopheline Mosquitoes*

The records of experiments of this nature do not comprise numerous observations, and in some cases the data given are insufficient for our present purpose. In Table III are shown the results of some experiments in which more complete data are given by the observers.

TABLE III.

Plasmodium falciparum, incubation period following the bites of anophelines previously infected.

Observer	Anopheline used	Wild or Bred	Healthy individual				Temperature at which mosquitoes kept	Remarks
			Number of mosquitoes fed	Number of days they were fed	Fever commenced in days	Parasites appeared in days		
Bastianelli and Bignami (1899)	*A. maculipennis* ...	Wild ...	3–2	2	9–12	10–13	30° C.	The person on whom the mosquitoes were infected developed Simple Tertian Malaria in 17–19 days.
Schüffner (1901) ...	*A. vagus*	Wild ...	?	2	15–16	17–18	Room	Large rings found.
Jancsó (1908) ...	*A. maculipennis* ...	Wild ...	2	1	12	12	24° C.	Quinine. 1 gm. on 7th day after bites.
Jancsó (1908) ...	*A. maculipennis* ...	Wild ...	1	2	10–15	11–16	20° C.	Quinine given after first bite, 1 gm., 1 gm., 1·5 gm., 1 5 gm., on 8th, 9th, 10th, 11th days respectively.
Mitzmain (1916) ...	*A. quadrimaculatus*	Bred ...	1	1	10	10	21–22° C.	Accidental infection.

In these experiments the maximum parasitic incubation period of *P. falciparum* after infection by means of bites of infective anophelines is eighteen days, the minimum ten days.

Before accepting the results of these observers, however, it is necessary to enquire more closely whether they are justified in regarding the experiments as conclusive. In Bastianelli and Bignami's experiment wild *A. maculipennis* were fed, for the purpose of infecting them, on a patient suffering from malignant tertian fever; this patient after seventeen to nineteen days from the commencement of feeding the mosquitoes on him showed in his blood the parasites of simple tertian fever. If the wild mosquitoes

infected him with *Plasmodium vivax* it appears not improbable that they might also infect with *P. vivax* the healthy person on whom they fed, and, therefore, one would expect that corroborative evidence such as the date of appearance of crescents in this case should be given. Apart from this, there is evidence, in some of these records, that the population used for experiment was subject to multiple infection. Reference has already been made to the fact that Gualdi and Antolisei, using the blood of patients supposed to be carrying *P. malariae*, obtained in the person injected *P. falciparum* This may be capable of many explanations, even excluding Laveran's theory, recently revived by Grassi (1920), of the unicity of the parasite, for example that they conveyed the parasite of malignant tertian fever by the inoculation, or that the person inoculated was himself already suffering from infection with *P. falciparum* or that he acquired such infection from mosquitoes independently of the inoculation. In the same way, in Bastianelli and Bignami's case, additional evidence as regards the species of parasite recovered is not available. In Jancsó's experiments the administration of quinine may have influenced the incubation perod. Mitzmain's accidental case appears to be the most satisfactory.

These considerations are mentioned in order to draw attention to the limited amount of reliable information available concerning the incubation period of *P. falciparum*. Factors such as the use of wild mosquitoes, experimenting among an already infected population, and the administration of quinine after the infective bite reduce materially the value to be attached to the figures. A further point to be mentioned, which may seriously affect the parasitic incubation periods given above, is that, as noticed previously, parasites are frequently present in relapse cases before a temperature reaction occurs. The importance of remembering this fact is seen when we consider Tables I, II and III. It will be noted that in these experiments, fourteen in number, parasites were always discovered either after the day of fever or on the day of fever, with the exception of one case (di Mattei), in which they were found 'a few days after injection.'

From what has been said, therefore, it is seen that the incubation period of malignant tertian malaria is a subject which would repay

further study. At present the evidence points to a minimum incubation, for inoculation of two days, and for mosquito bites of ten days. In view of the absence of evidence of inoculation infection in this case, we must suppose that an anopheline was the means of infection. There appears to be no sufficient evidence to exclude the possibility of a more brief incubation than ten days, the more so when we consider that a formerly accepted minimum incubation period of ten days for blood inoculation has given place to a later minimum incubation period of two days.

III. AT WHAT TIME AND WHERE DID THE CASE BECOME INFECTED?

It has been necessary to deal with a portion of this question above in discussing incubation periods, but there are other facts which may be employed to throw light on the subject.

Some assistance may be obtained from a study of the crescents and sporulating forms. Thomson (1911) states that, as a general rule, crescents appear in the peripheral blood on the fifth day after the attack of fever.

Marchiafava and Bignami (1894) found crescents in the finger blood of thirteen primary cases, usually between the seventh and eighth day of the disease, rarely as soon as on the fifth. Bignami and Bastianelli, who examined cases by spleen puncture, found crescents in the spleen as early as the seventh and eighth day, and exceptionally also in the peripheral blood.

It is seen from these observations that the spleen did not contain crescents any earlier than the peripheral blood. This corresponds with the findings in the present case, because an examination of smears of peripheral blood, spleen and bone marrow showed that whereas crescents were scanty in both peripheral blood and spleen, they were very numerous in the bone marrow. From this fact alone it could be deduced that death had occurred too early for crescents to be liberated from the bone marrow in large numbers. It has been shown by Marchiafava and Bignami (1894) that in certain cases of fatal malignant malaria, crescents may be scanty in the peripheral blood, spleen, and other viscera, while a very large preponderance of them is found in the bone marrow, where they may be seen in all stages. They conclude that the bone marrow

forms, if not the only, at least the most suitable site for the growth of crescents. They compare the passage of crescents from the bone marrow, where they are formed, with the corresponding passage of nucleated red cells from the bone marrow where they also are usually generated, into the general circulation.

Dudgeon and Clarke (1918-19) did not find crescents in their cases; they state in general of their fifty-one cases that no histological changes worthy of special reference were noted in the thyroid, bone marrow, testicles and pituitary body. They cite a very acute case of the haemorrhagic type, but no special mention of the condition of the bone marrow is made; it is a significant fact, however, that this case died in three days.

Applying the crescent periods and incubation periods to the present case we have a sequence, tracing back from the day of death, as follows:—October 12th, day of death, crescents present in small numbers in blood; allowing five days for crescents to appear in peripheral blood after paroxysm gives October 8th as day on which in a normal case the paroxysm should have occurred; allowing four days before this as the time when sporulating parasites first appeared in the peripheral blood, brings us to October 4th. From September 27th to October 4th is left for *parasitic* incubation period on the theory that she became infected by mosquito bite in the health resort in which it is known she was exposed to anophelines. This *parasitic* incubation period (seven days) is, even on the figures derived from the available experimental evidence, a probable one, and the history of the case supports it. It should be recalled that in this patient no shivering or sweating occurred and that we are dealing with a case of poor physique and considered to have been anaemic, the effect of which conditions upon incubation and reaction may be highly important, although by no means sufficiently recognised. Incubation will necessarily vary with the rapidity of the cycle of development of the parasite, the special reproductive energy of the particular parasite involved and the capacity of the infected human organism to facilitate or impede such parasitic reproduction.

In the light of our present knowledge of incubation periods, the occurrence of a mosquito infection six years ago or an inoculation infection eight months ago seems improbable, and we have further

no evidence at present to warrant us in regarding the case as one of endemic malaria. We are, therefore, compelled to decide whether she was infected at Liverpool or in the resort referred to. The evidence appears to me to support the last named as the place of infection.

THE SIGNIFICANCE OF CRESCENTS

Thomson (1911) considers the development of immunity to be necessary before crescents can be produced, but he thinks that they arise from ordinary merozoites, the period required for full development from merozoites to crescents being about ten days. He found, in common with previous observers, that crescents appeared in the blood about five days or more after the paroxysm, but while accepting the existence of a condition of immunity as essential to their production, he does not attribute their delayed appearance to the lapse of time required before immunity is established but simply to the length of time required for their development. The idea that the delay was due to the time required for immunity to establish itself had been put forward by Marchiafava and Bignami (1894) partly and also by Stephens and Christophers (1908). From a study of the appearances in this case, it is clear that the crescent-producing process was more advanced in the bone marrow than in the spleen or blood, and if the crescent-producing process is an 'immunizing' one, we may safely assume that its effect was first felt by the parasites in the bone marrow. Some evidence which might support the view that the process is connected with immunization may be obtained from a comparison of the relative numbers of crescents and segmenting forms present in the peripheral blood, spleen and bone marrow, respectively. In the peripheral blood the number of crescents and segmenting forms was about equal, in the spleen the segmenting forms far exceeded the crescents (fifteen to one), while in the bone marrow the segmenting forms were far outnumbered by the crescents (one to ten). Unless we assume that segmentation occurs at different hours in the bone marrow and spleen, or that red corpuscles containing young trophozoites cannot enter the bone marrow, on neither of which points is there any evidence, we are compelled to conclude that some agency in the bone marrow which causes the production of crescents

also prevents the completion of segmentation; that is to say, the bone marrow is the seat of a process which hinders the asexual while it facilitates the sexual development.

The number of crescents produced in any area such as the bone marrow which contains only a proportion of the total blood must be so small relatively to the areas where segmentation is occurring freely, that it seems evident that a certain time must elapse before the crescents reach the visibility point in the peripheral blood. This, in my opinion, is the real cause of the delay in their appearance, not only in primary cases but in relapses, and the delay seems bound to occur, even supposing that the 'immunizing' process already exists or becomes operative immediately in the bone marrow or other organs. It appears that this delay must occur, and that it is not necessary to attribute it to a growth period of the parasite, of the duration of which growth period this very delay is the only evidence. In this case the 'immunizing' process had commenced in the bone marrow, but there was no evidence, as indicated by great crescent production, that it had commenced in the other tissues available for examination. It is possible that in other cases it may occur early in other organs also.

NATURE OF THE 'IMMUNIZING' PROCESS WHICH RESULTS IN THE PRODUCTION OF CRESCENTS

Nothing definite can be deduced as to what this process is or what are the factors which call it into operation. But that it is in many cases quickly operative in the bone marrow is obvious, not only from this case but also from the observations mentioned above of the early appearance of crescents in bone marrow. It is also clear that many primary cases in which this process is already established in the bone marrow, nevertheless die, which supports the view that if the process is an 'immunizing' process it is focal in origin.

That the process is initiated by the mere presence of, or rupture of, segmenting forms, or by the toxins or pigment liberated at sporulation, is unlikely, in view of the observation in this case that in the spleen, where the most numerous segmenting forms occurred, crescents were scanty.

SUMMARY

1. A patient who had never been out of the British Isles died of malignant tertian malaria in Liverpool on October 12th, 1920.

2. There is evidence that this was an acute primary attack.

3. There is no evidence to show that this case acquired infection by other means than mosquito bite.

4. From a consideration of the records of incubation period and crescent formation, it is probable that the infection was acquired on or about September 27th, on which occasion she was in a northern health resort where anophelines are plentiful (*A. maculipennis*, *A. bifurcatus* and *A. plumbeus*).

5. Some evidence is given that crescent formation commences in the bone marrow, and that it is accompanied there by a failure of complete development in the asexual forms.

6. The 'immunizing' process which causes the above effects in all probability commences in some cases very early in the infection, but the crescents indicative of this process do not appear in the peripheral blood for some time.

7. The late appearance of crescents in the peripheral blood in infection with *P. falciparum* is explained on the ground that the source from which they arise is limited in extent.

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